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Overview of Reproductive and Developmental Toxicity Studies of 1,3-Butadiene in Rodents

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A series of studies to further evaluate the developmental and reproductive toxicity of inhaled 1,3-butadiene was sponsored by the National Toxicology Program. Pregnant Sprague-Dawley rats (24–28/group) and Swiss (CD-1) mice (18–22/group) were exposed to atmospheric concentrations of 0, 40, 200, or 1000 ppm 1,3-butadiene for 6 hr/day on days 6 through 15 of gestation (dg) and killed on dg 18 (mice) or dg 20 (rats). Subsequently, the uterine contents were evaluated; individual fetal body weights were recorded; and external, visceral, and skeletal examinations were performed. In rats, maternal toxicity was observed in the 1000-ppm group in the form of reduced extragestational weight gain and, during the first week of treatment, decreased body weight gain. Under these conditions, there was no evidence of developmental toxicity in rats. In contrast, results of the mouse developmental toxicity study indicated that the fetus may be more susceptible than the dam to inhaled 1,3-butadiene. Maternal toxicity was observed in mice at the 200- and 1000-ppm 1,3-butadiene exposure levels, whereas 40 ppm and higher concentrations of 1,3-butadiene caused significant exposure-related reductions in the mean body weights of male fetuses. Mean body weights of female fetuses were also reduced at the 200- and 1000-ppm exposure levels. No increased incidence of malformations was observed in either study. Other studies addressing male reproductive and mutagenesis end points were performed with B6C3F$_1$ mice (sperm-head morphology) and Swiss (CD-1) mice (dominant lethal study). In both studies, groups of 20 male mice were exposed to atmospheric concentrations of 0, 200, 1000, or 5000 ppm 1,3-butadiene 6 hr/day for 5 consecutive days. There were small concentration-related increases in abnormal sperm morphology 5 weeks following exposure (the only time of examination). Sequential postexposure examinations to determine the effect of 1,3-butadiene on all stages of gamete development were not performed. In the dominant lethal study, there were indications that exposure of males to levels as low as 200 ppm 1,3-butadiene caused an increase in the percentage of females with two or more dead implants in the first week following exposure. In both the first and second weeks following exposure, there were increases in the number of dead implantations (early), although strict atmospheric concentration-response relationships were not observed. These results suggest that more mature cells (spermatozoa and spermatids) might be adversely affected in this strain of mouse.

Introduction

1,3-Butadiene is a colorless, flammable gas that is produced from ethanol, butane, or butylene, or by catalytic cracking of light oil or naphtha (*1*). 1,3-Butadiene does not occur naturally. It is used in the preparation of a variety of chemicals and is the major component for the production of synthetic rubber; often in combination with styrene. 1,3-Butadiene is highly reactive, dimerizes to 4-vinylcyclohexene, and polymerizes easily. Because of this reactivity, inhibitors are added for its storage and transport. The major manufacturers of 1,3-butadiene in the U.S. produced about 1.5 million tons in 1980 (*2*).

Previous toxicity studies indicate that chronic inhalation exposure to 1,3-butadiene has the potential to affect the reproductive system. In a chronic toxicity and carcinogenicity study in rats (*3*), there was a significant increase in the incidence of mammary tumors in female

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rats and severe kidney toxicity in male rats after 24 months of exposure to 1000 or 8000 ppm of 1,3-butadiene. In this study, there was an increased incidence of several different tumor types, including those of the reproductive tract (Leydig cell tumors of the testes and uterine/vaginal stromal tumors). A chronic toxicity and carcinogenicity study in which mice were exposed to 625 or 1250 ppm of 1,3-butadiene was terminated at about 60 weeks of exposure because of high mortality associated with neoplasms at multiple sites, including ovarian granulosa cell tumors (*4*). There was also a significant increase in the incidence of mice with testicular or ovarian atrophy.

Two reports in the literature present information regarding the potential of 1,3-butadiene to cause reproductive or developmental toxicity in laboratory animals. In a reproductive study, male and female rats were exposed to 600, 2300, or 6700 ppm of 1,3-butadiene for 8 months (*5*). A concentration-related depression of body weight was observed. Reproductive studies of male and female animals were performed at unstated times during these exposures, but no data were given concerning the number of matings or of barren females. The number of litters from female rats in all exposed groups tended to be lower than in controls, although litter sizes were stated to slightly exceed the expected norm of six per litter. Limited breeding tests of the offspring of the 2300- and 6700-ppm exposure groups suggested that there was reduced fecundity, but it was not determined whether the deficit was associated with the males or the females. Although details were not provided, a small number of guinea pigs and rabbits was also used in each exposure group for reproductive studies. Animals of all groups evidently produced progeny, except for rabbits exposed to 600 and 2300 ppm. The basis for the authors' conclusion that any apparent reduction in fertility was due to hereditary characteristics is not clear.

A teratology study has been reported (*6*) wherein female Sprague-Dawley rats were exposed 6 hr per day to 0, 200, 1000, or 8000 ppm of 1,3-butadiene from days 6 through 15 of gestation. There was a significant concentration-related suppression of maternal body weight gains during exposure, but body weight gains during gestation (adjusted for conceptus weight) were significantly depressed only in animals exposed to the two highest concentrations. Reproductive measures (pregnancy rate, gravid uterus weight, number of implantation sites, number of fetuses per dam, and preimplantation loss) were not affected by 1,3-butadiene exposure. Mortality of postimplantation embryos tended to be slightly higher in all groups of exposed animals than in controls, but the difference was statistically significant only at the highest concentration.

Body weights and crown-rump lengths of fetuses of the highest exposure group were significantly less than those of control fetuses (*6*). There was a significant increase in minor fetal defects (hematomas and minor skeletal defects) in all groups, and exposure to 1000 or 8000 ppm resulted in significantly higher incidences of major skeletal defects. In the 8000-ppm group, there were significant increases in the incidence of other anomalies including lens opacities and irregularities of ossification. The incidence of wavy rib, which was rarely observed in historical controls at this laboratory, was 1.6% in the control animals for this study and increased in a concentration-related manner for exposed animals. The authors concluded that this response was not indicative of a teratogenic effect, but was due to maternally mediated embryonic growth retardation.

The genetic toxicity literature on 1,3-butadiene has been recently reviewed (*7*). A study using male $B6C3F_1$ mice exposed to 6.25, 62.5, or 625 ppm 1,3-butadiene for 6 hr/day for 10 days over a 2-week period revealed significant increases in the frequency of chromosomal aberrations and sister chromatid exchanges, lengthening of average generation time, and a depression of the mitotic index in bone marrow cells (*8*).

Based on the large volume of production of 1,3-butadiene and the toxicity data available that indicated the potential of this chemical to affect the development of the conceptus as well as the structure and function of reproductive organs, a series of studies was conducted to evaluate in greater depth the potential of 1,3-butadiene to cause developmental toxicity in rats and mice and to cause mutagenic changes in dominant lethal and sperm-head morphology studies. This paper summarizes the results of these four toxicity studies.

Methods and Materials

1,3-Butadiene was obtained from the Phillips Chemical Company (Borger, TX). For this series of studies, one lot (F909) of 1,3-butadiene was used, the identity of which was confirmed by infrared spectroscopy. Gas chromatograph analysis (2 m × 2 mm Porapak QS 100/120, 100°C isothermal, flame ionization detector) for area percent purity of 1,3-butadiene determined the purity to be 99.88%. Since the rate of polymerization of 1,3-butadiene is known to be temperature dependent, care was taken to assure constant storage temperatures of approximately 72°F. The cylinders of 1,3-butadiene were never emptied beyond 80% of their volume to prevent the appearance of high concentration of the dimer in the 1,3-butadiene atmospheres. Cylinders of 1,3-butadiene were not used if the dimer concentration in the head space exceeded 500 pm.

Animals for these studies were obtained as follows: Sprague-Dawley rats for the teratology study, $B6C3F_1$ mice for the sperm-head morphology study, and Swiss (CD-1) mice for the dominant lethal study were obtained from the Charles River Laboratory at Portage, MI. The Swiss (CD-1) mice for the teratology study were obtained from the Charles River Laboratory at Kingston, NY. These species and strains were selected because of historical use by the National Toxicology Program (NTP). All animals were sexually mature when obtained and were acclimated to the laboratory for a minimum of 14 days prior to their random assignment to treatment

Table 1. Experimental design for teratology study of rats and mice exposed to 1,3-butadiene.

Parameter	Mice	Rats	Mice and rats
Chamber concentrations, ppm	—	—	0, 40, 200, 1000
Number of sperm-positive females per exposure group	32, 33, 31, 33	30	—
Number of days mating (resulting in five exposure subgroups)	—	—	5
Exposure regimen	—	—	6–15 dg,[a] 6 hr/day
Day of sacrifice	18 dg	20 dg	—
Maternal observations	Body weights on 0, 6, 11, 16, and 18 dg	Body weights on 0, 6, 11, 16, and 20 dg	Signs of toxicity
	—	—	Lesions at gross necropsy, weight of gravid uterus, number of implantation sites, intrauterine mortality, and placental weights
Fetal observations	—	—	Body weight tabulated by sex; gross, visceral, and skeletal exams

[a]dg = days of gestation.

groups within studies. Animals were randomized by weight to minimize potential differences between groups at the initiation of exposures. All animals were identified by ear tags and were observed daily throughout the study for signs of toxicity. Animals were housed in sanitized, stainless-steel, wire-mesh cages. All animals were fed NIH-07 open formula diet *ad libitum* during nonexposure hours. Water was available *ad libitum* through automatic watering systems. The animals were maintained under conditions designed to provide a temperature of 72 ± 3°F and relative humidity of 50 ± 15%. All animals were on a 12-hr light-dark cycle.

Experimental Design

Developmental Toxicity Studies

The design of the teratology studies of rats and mice exposed to 1,3-butadiene is summarized in Table 1. All animals for these studies were observed twice daily for mortality, morbidity, and signs of toxicity. Females were weighed during the week prior to mating and on days 0, 6, 11, and 16 of gestation. On gestation day 18 (mice) or 20 (rats), the animals were killed with CO_2, weighed, and examined for gross tissue abnormalities. The uterus was removed and weighed; apparent nongravid uteri were stained with ammonium sulfide to detect implantation sites (*9*). The number, position, and status of implants were recorded for gravid uteri; placentas were examined and weighed. Live fetuses were weighed, examined for gross defects, and their sex was noted. Examinations for fetal lens opacities were conducted by removing the eyelid and examining the eye *in situ*. In addition, the eyeballs were removed for observation under the dissecting microscope. Visceral examinations (*10*) and skeletal examinations, using specimens stained with alcian blue and alizarin red (*11*), were performed on all live fetuses. Approximately 50% of the fetal heads were examined by razor blade sectioning of fixed preparations (*12*).

Table 2. Experimental design for the sperm morphology study of mice exposed to 1,3-butadiene.

Parameter	Specifications
Chamber concentration	0, 200, 1000, and 5000 ppm
Exposure regimen	5 successive days, 6 hr/day
Number of males/group	20
Time of sacrifice	During postexposure week 5.
Observations	Signs of toxicity
	Body weights prior to experimental treatment and 0, 1, 2, 3, 4, and 5 weeks after treatment
	Examination of epididymal preparations for spermhead abnormalities

Sperm-Head Morphology Assay

The experimental design of the sperm-head morphology study is summarized in Table 2. The animals in this study were observed twice daily for mortality, morbidity, and signs of toxicity; body weights were determined at weekly intervals. During the fifth post-exposure week, the mice were killed with CO_2, weighed, and examined for lesions of the reproductive tract and for gross tissue abnormalities. The cauda of the right epididymis was removed and processed to obtain the sperm suspension. Sperm suspensions were stained with 1% eosin Y and 2 to 3 drops were transferred to microscope slides (four slides per mouse). The suspensions were dried and mounted under coverslips with mounting medium. The evaluation of sperm heads was done in a blind manner. The morphology of at least 500 sperm heads from each mouse was categorized as normal or abnormal (blunt hook, banana, amorphous, pinhead, two heads/two tails, short, and any other type of variants encountered during the examination).

Dominant Lethal Study

The design of the dominant lethal study is summarized in Table 3. The animals were observed twice daily for mortality, morbidity, and signs of toxicity; body weights

Table 3. Experimental design for the dominant lethal study of mice exposed to 1,3-butadiene.

Parameter	Specifications
Chamber concentration	70, 200, 1000, and 5000 ppm
Exposure regimen	5 successive days; 6 hr/day
Number of males/group	Cohabitation of each male with two females for 1 week; matings for 8 weeks with replacement of two females each week
Time of sacrifice	Females: 12 days after last day of cohabitation with 1,3-butadiene-exposed males Males: after week 8 of mating
Observations	Females: reproductive status; total number, position, and status of uterine implants Males: signs of toxicity—body weights before experimental treatment and each week for 8 weeks following treatment; examination of gross tissue abnormalities.

of the males were determined at weekly intervals. Following the eighth week of cohabitation with groups of untreated females, male mice were killed with CO_2, weighed, and examined for lesions of the reproductive tract and gross tissue abnormalities. Female mice were killed with CO_2 12 days after the last day of cohabitation. The reproductive status was determined and the gravid uterus was removed to determine the total number, position, and status of implantations. The numbers of early and late resorptions and live and dead fetuses were determined.

Inhalation Exposures

All animals were exposed to the test atmospheres within Hazelton 2.3 m^3 stainless-steel inhalation chambers. Animals were exposed in individual cages equipped with feed troughs and automatic watering devices. During exposure, the feed troughs were removed from each cage unit. Water was available *ad libitum* at all times.

For generation of chamber atmospheres, 1,3-butadiene was withdrawn directly from a gas cylinder and diluted with filtered air. An air-driven vacuum pump delivered the 1,3-butadiene-air mixture to the exposure chamber inlet for final dilution to the desired concentration.

The concentration of 1,3-butadiene in the chambers was monitored using an HP 5840 gas chromatograph equipped with a flame ionization detector (oven, 120°C; ⅛ × 12″ nickel column packed with 1% SP-1000 on 60/80 mesh Carbopack B). The mean daily concentration of 1,3-butadiene in the chambers during these studies never deviated from the intended concentration by more than 10%. The highest relative standard deviation for any one of the exposure groups during these studies was 3%. Thus, because of the extreme closeness of the analyzed concentration to the intended nominal concentrations, reference throughout this paper is made only to the nominal concentrations.

Statistical Evaluation

In the teratology studies, analysis of variance was used to analyze weight data and, if the result of the analysis was significant, Duncan's multiple range test was performed to delineate intergroup differences. Response proportions such as the number of resorptions; implants; and live, dead, or affected fetuses per litter were also analyzed by an analysis of variance following arcsin transformation of the response proportion. Binary-response variables between groups was compared, using chi-square or Fisher's exact test (*13*), e.g, numbers of pregnant females per number inseminated.

In the dominant lethal study, the number of implantation sites and intrauterine deaths per litter for each week were analyzed by analysis of variance. When appropriate, proportions of resorptions and dead or live fetuses per implant were subjected to an arcsin transformation and evaluated by analysis of variance. If the analysis of variance F statistic was significant, Duncan's multiple range test was used to delineate intergroup differences.

In the sperm-head morphology study, values for normal and abnormal sperm heads were expressed as a percentage of the total number of cells examined for each animal. These data were subjected to an arcsin transformation and evaluated by an analysis of variance. If the analysis of variance F statistic was significant, Duncan's multiple range test was used to delineate intergroup differences. Dose response trends were determined by means of orthogonal contrast (*14*).

Results

Developmental Toxicity Studies

In the rat study, the only toxicity observed was in dams at the 1000 ppm exposure level (*15*). There was a decrease in maternal body weight gain between days 6 and 11 of gestation (dg) and a decrease in extragestational body weight gain (dam body weight on dg 20 minus weight of the gravid uterus). The percentage of pregnant animals and number of litters with live fetuses were unaffected by treatment. Similarly, there were no significant differences among exposure groups with respect to number of live fetuses per litter, percentage resorptions or percentage malformations per litter, placental or fetal body weights, or sex ratio.

In the mouse study, measures of maternal toxicity were adversely affected by 1,3-butadiene exposure during pregnancy (*16*) (Table 4). As in the rat study, extragestational weight gain was reduced, but in this study it was also reduced at 200 ppm as well as 1000 ppm. Maternal body weight gain from dg 11 to 16 was also adversely affected at these exposure levels. In addition, maternal body weight on dg 18 and gravid uterine weight were reduced in the 1000-ppm group relative to the control group. Maternal body weight gain was not reduced during other periods of gestation. Fetal and placental weights were reduced in an exposure-related

Table 4. Maternal and developmental toxicity in Swiss (CD-1) mice resulting from exposure to 1,3-butadiene.

Parameter	Atmospheric concentration of 1,3-butadiene, ppm 40	200	1000
Maternal body weight, 18 dg			↓[a]
Maternal weight gain, 11–16 dg		↓	↓
Extragestational weight gain[b]		↓	↓
Gravid uterine weight			↓
Fetal body weight		↓	↓
Females		↓	↓
Males	↓	↓	↓
Placenta weight		↓	↓
Females			↓
Males		↓	↓

[a]Arrows denote a statistically significant ($p \leq 0.05$) decrease compared with the control group value.

[b]Weight gain of the dam corrected for the weight of the gravid uterus.

Table 5. Results of dominant lethal study in Swiss (CD-1) mice exposed to 1,3-butadiene for 5 consecutive days.

Parameter	Atmospheric concentration of 1,3-butadiene, ppm 200	1000	5000
Week 1			
Dead implants/total implants, %		↑[a]	
Females with ≥ 2 dead implants, %	↑	↑	
No. dead implants/pregnancy		↑	↑
Early		↑	
Late			
Week 2			
No. dead implants/pregnancy	↑	↑	
Early	↑	↑	
Late			

[a]Arrows denote a statistically significant ($p \leq 0.05$) increase compared with the control group value. The absence of an arrow denotes no difference from control.

manner. At 40 ppm, mean male fetus body weight was reduced to 95% of the control value. A similar decrease in female fetus body weight was not statistically significant at this level, but values for both male and female fetal body weights were significantly reduced at the 200- and 1000-ppm 1,3-butadiene levels. Placenta weights of male and female fetuses were reduced at the 1000-ppm 1,3-butadiene concentration, but only placenta weight of male fetuses was reduced at 200 ppm 1,3-butadiene. There were no significant differences in percentage resorptions or percentage malformations per litter, although increases in fetal variations (supernumary ribs, reduced ossification of sternebrae) were observed at exposure levels of 200 and 1000 ppm.

Sperm-Head Morphology Assay

There was no mortality as a result of treatment of male $B6C3F_1$ mice at 200, 1000, or 5000 ppm for 5 consecutive days (*17*). Transient toxic signs (piloerection and dyspnea) were observed for 20 to 30 min following exposure to 5000 ppm 1,3-butadiene. Mean body weights of 1,3-butadiene-exposed groups were not significantly different from the control value. There were concentration-related small increases in the percentage of abnormal sperm heads at the 1000- and 5000-ppm 1,3-butadiene levels at 5 weeks after treatment (other time points were not evaluated). There was a 21% increase in abnormal sperm at 200 ppm (not statistically significant), 73% at 1000, and 129% at 5000 ppm. The background incidence of abnormal sperm in the control group (1.6%) is consistent with the NTP historical control value (1.7%) for this strain of mouse.

Dominant Lethal Study

There was no mortality with CD-1 mice using concentrations of 1,3-butadiene up to 5000 ppm for 5 consecutive days (*18*). Transient toxic signs were similar to those in the sperm-head morphology assay, and body weights were unaffected by treatment.

Week 1. At 1000 ppm (week 1 following exposure) there was a significant increase in the percentage of dead implants expressed as a function of total implantations (Table 5). There were smaller increases at 200 and 5000 ppm that were not statistically significant compared with the control group. Of the female mice mated with exposed males, there were significant increases (approximately 3-fold) in the percentage of females with two or more dead implantations in all 1,3-butadiene-mated groups. The number of dead implantations per pregnancy was increased only in the 1000-ppm group.

Week 2. During the second week following exposure, the number of dead implantations per pregnancy was increased in both the 200- and 1000-ppm groups, but not in the 5000-ppm 1,3-butadiene group. No significant increases in the end points evaluated were observed in weeks 3 to 8. While not strongly concentration-dependent, these results are consistent with an adverse effect of 1,3-butadiene on more mature cells (spermatozoa and spermatids).

Discussion

The lack of developmental toxicity in the current rat study is in contrast to the results of a prior study (*6*) that reported an increase in major skeletal defects (as a percentage of the fetuses evaluated) in the 1000- and 8000-ppm 1,3-butadiene exposure groups. The values are similar if expressed as percentage skeletal malformations per litter (0.6, 2.5, 2.8, and 6.0%, control, 200, 1000, 8000 ppm levels, respectively). Both studies detected a decrease in extragestational weight gain of dams exposed to 1000 ppm 1,3-butadiene. The earlier study also detected a concentration-related decrease in maternal weight gain during the exposure period that extended to the 200-ppm group (the lowest level tested); no similar decrease was observed at the 40- or 200-ppm levels in the current study. The basis for the discrepancy in maternal and embryo-fetal effects is not known.

Although developmental toxicity was not observed in the present rat study, there may be reason for concern

for the developing organism based on results of the mouse study reported here. In this study, maternal toxicity was observed at the 200- and 1000-ppm 1,3-butadiene levels, whereas developmental toxicity in the form of reduced male fetal body weight was present at the lowest level tested, 40 ppm 1,3-butadiene. Since developmental toxicity, which can be manifested as malformation, intrauterine death, decreased body weight, or functional deficits, may be expressed in different ways in different species, the decrease in male fetal body weight, particularly in the absence of maternal toxicity, may be of concern considering that the Occupational Safety and Health Association (OSHA) standard for 1,3-butadiene is currently 1000 ppm.

Thus, no developmental toxicity was seen in rats at any of the concentrations tested, even in the presence of maternal toxicity (1000 ppm). These studies showed developmental toxicity in mice in the presence of maternal toxicity (200 and 1000 ppm 1,3-butadiene) and decreased fetal body weight in the absence of maternal toxicity (40 ppm). Sperm-head morphology and dominant lethal studies in mice given concentrations of 1,3-butadiene between 200 and 5000 ppm showed responses that may be indicative of weak germ cell mutagenic activity. The possibility of germ cell effects is supported by clear evidence of genotoxicity in somatic cells, as indicated by significant increases in sister chromatid exchange at a concentration of 6 ppm 1,3-butadiene, as well as by other genotoxicity assays (*8*).

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